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COMPARISM OF THE PROPERTIES AND YIELD OF BIOETHANOL FROM MANGO AND ORANGE WASTE

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ABSTRACT

The excessive consumption of fossil fuel particularly in urban areas due to transportation and industrial activities has greatly contributed to generation of high levels of pollution; therefore, a renewable eco-friendly energy source is required. The production of bioethanol from sugar extracted from waste fruit peels as an energy supply is renewable as the non-fossil carbon source used is readily replenished. Laboratory experiments were conducted to evaluate the chemical composition of fruit wastes of orange and Mango in order to explore their potential application in bio-ethanol production. Experimental production of Bioethanol from waste fruits of mango and orange was carried out after dilute acid pretreatment followed by enzymatic saccharification using saccharomyces cerevisiae for the fermentation process. Three samples of (mango waste fruit, orange waste fruit and mixture of mango and orange waste fruit) 100g each was used for the same method of bio-ethanol extraction. A one factor factorial design involving fruit type was used to statistically analyze the fuel properties of the ethanol produced from the fruits waste. Analysis of variance (ANOVA) shows that the observed difference were not significant for all the properties except that of the flash point which showed that the flash point of the produced bioethanol differ from that of the standard ethanol, which may be due to percentage of moisture present in the samples used. The highest yield of ethanol from sample A (mango waste) was 19.98%, sample B (orange waste) produced 19.17% while least yield of ethanol was from sample C (mango and orange waste) which produced 17.38%.

Key words: fossil-fuel, pollution, renewable, bio-ethanol, yield

1.0 Introduction

The use of "gasohol" (a blend of gasoline and bioethanol) as fuel for cars is perhaps the best-known example of the large-scale use of renewable fuels. This product is sold in sixteen countries and ten states in the USA (Celic, 2008). Bioethanol is obtained by fermentation of renewable sources for fuels or fuel additives, due to gradual decrease of fossil fuels; bioethanol has gotten the attention of many researchers to use it as an alternative source of energy across the world. There is also the need to find environmentally sustainable energy source as a viable long-term substitute for liquid petroleum, as a step to solve the problem of sustainability and pollution the use or addition of bioethanol to gasoline in the internal combustion engines reduces the emission of carbon monoxide (CO) and unburned hydrocarbon that form smog; this has widely been enforced in recent years so that the net emission of carbon dioxide will be close to zero (Wyman, 1994).

The biofuels industry has evolved from using first generation feedstock (typically food crops) to using second and third generation feedstock, for both bioethanol and biodiesel, Saratale et, al (2008). While the term bio-fuels denote any fuel made from biological sources, for most practical uses, the term refers to either bioethanol or biodiesel. The last few years have seen tremendous growth in bio-fuels. Converting a renewable non-fossil carbon, such as organic

wastes and biomass consisting of all growing organic matter (plants, grasses, waste fruits and algae) to fuel would ensure a continual energy supply (Wyman, 1994). According to the United State Department of Energy, for every unit of energy consumed for bioethanol production, 1.3 units of energy are returned Hil, *et, al.* (2006).

Agricultural waste from fruits like mango and orange are plant- based material that can be an ethanol feedstock. All plants contain sugars, and these sugars can be fermented to make ethanol in a process called biochemical conversion. Plant material can also be converted to ethanol using heat and chemicals in a process called thermo chemical conversion. These agricultural waste fruits (mango and orange) generate solid waste and economic losses to farmers both in the farm and the market places, therefore, the use of these waste for bioethanol production shall reclaim the farmers economic loss and rid the environment of the negative impact of these waste, Lo, *et al,(2009)*. The high rate of Nigeria's dependency on crude oil and the consequences of using fossil fuel (high rate of greenhouse gasses generation) require immediate attention with respect to the production of bioethanol for blending with fossil fuel in order to produce a much cleaner and eco-friendly fuel (lower rate of greenhouse gasses generation) for use in our machinery. Ethanol, also known as ethyl alcohol with the chemical formula C_2H_5: "The fuel of the future is going to come from fruit like (mango and orange waste) that's out by the road, or from apples, weeds, sawdust – almost anything that's plant based. There is fuel in every bit of vegetable matter that can be fermented. There is enough alcohol in one year's yield of an acre of potatoes to drive the machinery necessary to cultivate the fields for a hundred years, Saratale *et al.,* (2008). However, fossil fuels were pre-dominantly used for automobile transportation throughout the last century, obviously due to their lower production cost. As an automotive fuel, hydrous ethanol can be used as a substitute OH, is a flammable, clear, colorless and slightly toxic chemical compound with acceptable odour. It can be produced either from petrochemical feedstock by the acid-catalyzed hydration of ethene, or from biomass feedstock through fermentation. On a global scale, synthetic ethanol accounts for about 5-10% of total production while the rest is produced from fermentation of biomass mainly sugar crops, e.g. cane and beet, and of grains (mainly corn). Ethanol as a neat fuel or even in the blended form with gasoline has a long history as automotive fuel. In 1860, German inventor Nicholas Otto used ethanol as a fuel in an early prototype of an internal combustion engine because it was widely available throughout Europe for use in spirit lamps. A few years later, Henry Ford built his first automobile with an engine that could run on ethanol. In 1908, Ford unveiled his Model T engine equipped with carburetors that could be adjusted to use alcohol, gasoline or a mixture of both fuels. Ethyl alcohol as "the fuel of the future" was presented by him for the first time. Anhydrous ethanol, on the other hand, is an effective octane booster when mixed in blends of 5% to 30% with no engine modification requirement.

This study is aimed at producing bioethanol from waste fruits of Mango (*Mangifera indica*) and Orange (*citrus*) by fermentation process using rotten potatoes and tomatoes as vegetable medium (potatoes dextrose agar) to generate enzymes for the fermentation process; the extracted

bioethanol properties shall be compared with standard pure (98%) hydrous ethanol in order to identify the waste fruit with the highest yield.

2.0 Materials and Methods

The basic biology behind bioethanol production is the action of microorganism in the form of yeast anaerobic on sugar containing solution and its subsequent conversion into alcohol. The reaction involved can be represented by the following equations.

Sugar + yeast → ethanol + carbon dioxide……………………………………………..2.0

C_6H_{12} + yeast → $2C_2H_5OH + 2CO_2$ ………………………………………..………2.1

Starch with the chemical formula $C_6H_{10}O_5$ is first of all converted to glucose which is then fermented into bioethanol using the equipment and reagents mentioned in the chemical equations show these transformations as follows

Starch + water →glucose……………………………………………………….……….....2.2

$C_6H_{10}O_6 + H_2O \rightarrow C_6H_{12}O_6$……………………………………………….………..…2.3

2.1 Materials and equipment used

Waste fruit samples of equal weight (100g) of mango ( ) orange ( ) and equal proportion of the two samples put together as the third sample (mango and orange), were collected from gamborou market in Maiduguri (fruits major market), Borno state Nigeria. Waste potatoes and tomatoes for vegetable medium preparation were also collected at the same market. refractometer, autoclave machine, conical flasks, spatula, industrial oven, pycnometer, thermostat, test tubes, thermometer, flash point apparatus, distillation apparatus, pH paper and meter, dry active yeast, yeast extract, Sodium chloride, magnesium chloride, sodium nitrate and potassium chloride as mineral salt medium (MSM), lactic acid, lactic phenol and dextrose (for synthetic nutrient broth), sodium hydroxide for pH correction, sulphuric acid for hydrolysis, 98% pure ethanol (used to prepare standard curve for ethanol), de-ionized water, pipette, buffer solution, and U-tubes capillary viscometer.

2.2 Methods

2.2.1 Experimental procedure (Preparation and Sterilization of Growth Media)

All the glasses were put in a container with tap water and liquid wash was added and left to stay for 10-15 minutes and then washed with a clean water to remove all stains and impurities; finally they were rinsed with distilled water, and sterilized with hot air in an oven for 30 minutes at a temperature of $180^{O}C$ (Adney, 1996). 200 grams of potatoes was peeled, shed and boiled in a conical flask for 20 minutes, it was turned into another conical flask and filtered. The filtrate was mixed with dextrose. Another conical flask was boiled with water inside it and nutrient agar was poured little by little and stirred to avoid formation of lumps. The dextrose with the filtrate was mixed with agar solution (as our growth media). When a complete solution of potatoes dextrose agar (PDA) was achieved the conical flask was corked with a large amount of non-absorbent

wool to prevent contamination and then covered with aluminum foil. The flask was sterilized in a clinical autoclave for 20 minutes at a maximum temperature of 120°C and operating pressure of 15 Psi, Mamma *et al.*, (2008). The cotton plug was wrapped with aluminum foil to avoid possible moistening of the plugs by condensing steam. On attaining the required sterilization period (20mins) the clinical autoclave was switched off and cooled to room temperature. The sterilized and cooled medium was stored in a refrigerator.

2.2.2 Microorganism culturing and acidified solution for Pons culture

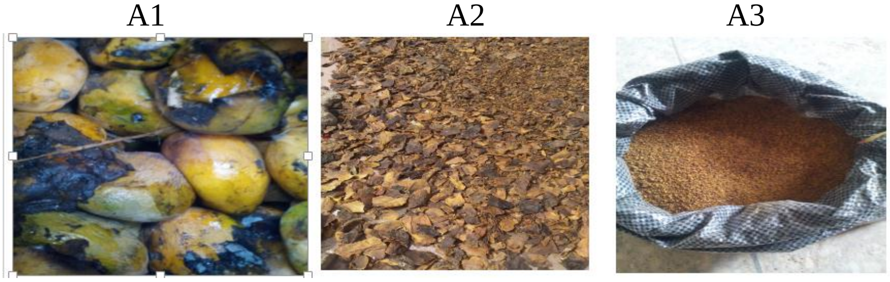
Lactic acid was prepared into a solution of 59 percent lactic acid with distilled water to prevent the activities of bacteria in the culture medium (Adney, 1996) The potatoes dextrose agar (PDA) medium already prepared in the clinical autoclave, was cooled to a temperature of 40^{o}C before it is poured into the sterilized Petri dishes, 59 percent of lactic acid was poured into each of the Petri dishes to acidify the dishes, the medium poured into the Petri dishes was allowed to cool to room temperature, solidify and ready for culturing. The inoculating loop was sterilized with alcohol (methylated spirit) and heated with spirit lamp until is partially red hot. The inoculating loop was first put in the acidified culture medium to reduce the needle temperature. The loop was then used to cut a portion of the Parent culture from visible matured strain, which is immediately put in the counter of the freshly acidified and cooled medium Ban-Koffi, *et al*, *(1990)* as shown in Figure 1.

Figure 1: The Pure culture preparation (Anas, 2015)

2.2.3 Sample collection and preparation

The sample of waste mango used for the extraction of bioethanol is shown in Figure 2. Where A1 is the raw mango, A2 is the dried peeled with pulp and A3 is the grinded rotten mango.

A1 A2 A3



Rotten mango peeled dried mango grinded mango

Figure 2: The mango sample

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The sample of waste orange used for the extraction of ethanol is shown in Figure 3. Where B1 is the raw orange, B2 is the dried peeled with pulp and B3 is the grinded rotten orange.

B1 B2 B3

Rotten orange peeled dried orange grinded orange

Figure 3: The orange sample

C1 C2

50% dried mango 50% dried orange

Figure 4: Ratio of sample A and sample B before mixing to form sample C.

2.2.4 Preparation of substrate

The choice of the substrate was 2 different varieties of waste fruits (mango and orange). The waste fruits was peeled, sliced in to pieces and allowed to sundry which is then grinded into powder form as in figures 2, 3 and 4. The powder form of waste fruits were weighed with electronic weighing machine (model mp 1002, made in Japan) into 3 different samples i.e. A, B and C, 100g of orange, 100g of mango and a mixture of orange and mango at equal proportions (fig. 4) 50g of each respectively which was put into transparent gallons, 600ml of mineral salt medium (MSM) was poured into each sample mixed and tight the gallons properly for fermentation to take place as shown in Figure 5.

Figure 5: The Substrate preparation (Anas, 2015)

2.2.5 Inoculating the substrate

The inoculating loop was sterilized with cotton wool soaked with 70% alcohol to destroy any existing contamination. A well developed colony of the already sub-cultured pure specie of saccharomyces cerevisiae was taken using the inoculating loop, which was dipped into the substrate and mixed properly, Manikandan, *et al*, *(2008)*. This process was repeated for the remaining samples inside the gallons.

2.2.6 Observation of activity of the microorganism

Observation of the activity of the microbes was done on a daily basis as the change in glucose concentration of the samples were measured and recorded in the three sample shown in plate 2.6. where anaerobic reaction/digestion is taking place.

Plate 2.6: The fermentation of three samples (Anas, 2015)

2.2.7 Tests for glucose

The glucose test was carried out using the Randox standard glucose kit. This was done first by preparing the sample, the standard before the analysis of the entire sample involved 4 ml of filtrate taken from the conical flask and transferred using a pipette into the test tube, 1 ml of the reagent is added into it. The test for glucose was carried out using spectrophotometer, 4 cubes were used, the first cube was used as a blank where only distilled water was poured and in the other 3 cubes, the samples solution weighing 100g each was poured unto the cubes inside the spectrophotometer machine and the amount of glucose was measured. The amount of glucose measured was directly proportional to the amount of bioethanol been formed in the sample solution, Reddy, *et al.*, (2011).

2.2.8 Determination of the properties of ethanol

2.2.9 Distillation range test

A known volume of the bioethanol was turned into ASTM distillation flask fixed at it position. A thermometer inserted at the top and switched on. A receiver (cylinder) was kept at the distillate recovery point. The temperature reading was taken at the first drop at the distillate. As the temperature increased, the distillate increased in volume until there was a decrease in distillate and at the last drop the final temperature and volume were noted, Adney, *et al., (1996).*

Appearance: the distillate was observed visually, using visual test method.

2.2.10 Ethanol yield

Bioethanol concentration was monitored following the method described by Adney, *et al.*, (1996) using refractive index method. The refractive index was determined with refractometer (Bellingham and Stanley, England 366A-3R). A calibration curve was obtained initially by diluting pure 98% ethanol in water to obtain different concentrations of bioethanol and their corresponding refractive index obtained from the refractometer, while percentage yield of each sample was determined by the formula; Yield point % = (sample weight/mass of sample distillate) × 100, Adney, *et al., (1996)*.

2.2.11 Flash point

This test was carried out using penskymartens flash point apparatus. The cup in the apparatus was dried. 50 ml of each sample (ethanol produced) was transferred into the position in the apparatus assembled with thermometer and the apparatus was switched on, the heat was controlled by a steady stirrer to maintain a uniform temperature while passing a small flame across the material every five seconds. The temperature at which the vapor first flashes with the blue flame was recorded as the flash point of the samples after each test cup was washed and dried before the subsequent test, Adney, *et al., (1996).*

2.2.12 PH test

The PH meter probe was first inserted in a buffer solution to standardize the apparatus then placed into the sample (bioethanol) and then the readings were obtained.

2.2.13 Density and specific gravity test

Empty pycnometer was weighed. The pycnometer was filled with sample (ethanol) the excess was wiped off, the weight was recorded and density is calculated using the formula

Density $(\frac{g}{ml}) = \frac{mass}{volume}$ or Density $= \frac{M2 - M0}{M1 - Mo}$

Where M_2 = mass of empty bottle in (g), M_1 = mass of empty bottle + water in (g)

M_0 = mass of the substance in (g)

Secondly distilled water was filled into the pycnometer, weighed and recorded. The specific gravity was calculated using the formula

Specific gravity (spg) = density of bioethanol/density of water.

2.2.14 Viscosity test

50 ml of bioethanol was turned into A-arm of tube capillary viscometer through the orifices to the marked point; a sucker was used to lift the sample to the b-arm of the capillary to the marked point. A stop watch was used to regulate the time it took the bioethanol to return (flow) to the mark under the B-arm and the time noted. Viscosity calibration curve was then used to convert viscosity in seconds to centistokes, Adney, *et al.*, (1996).

2.2.15 Boiling point

Boiling point is the temperature at which the vapor pressure is equal to atmospheric pressure and was determined by noting the reading of the inserted thermometer through the cork covering the conical flask containing the sample being heated.

3.0 Results and Discussion

The result in table 1 shows an increase in PH value for each different samples as fermentation increases, which also shows that the acidity concentration of all the samples are decreasing while fermentation rate increases since the PH value was moving towards basicity.

Table 1: pH values obtained from each sample during first week of fermentation

Time (hrs)	Sample A (mango waste fruits)	Sample B (orange waste fruits)	Sample C (mango and orange)
24	5	5.16	5.08
48	5.26	5.18	5.22
72	5.45	5.6	5.23
96	5.84	5.64	5.74
120	6.5	6.23	6.37
144	6.85	6.60	6.73
168	6.72	6.81	6.77

The result in table 2 shows that glucose concentration and absorbance from sample A increases more rapidly than the other sample at first week of fermentation, this because sample A has lower acid content that requires less time to digest and ferment subsequently as compared to orange with higher acid content and requires more time to digest before fermentation.

Table 2: Glucose test results obtained from each sample during first week of fermentation at intervals of two days.

Sample	Time (hrs)	Quantity (g)	Absorbance	Conc. Mol/L	Conc. %
A(mango)	48	100	1.983	1.991×10^{-3}	0.199
B(orange)	96	100	1.944	1.951×10^{-3}	0.195
C(mango-orange)	144	100	1.843	1.835×10^{-3}	0.184

The result of Table 3 shows that sample A has the least percentage of moisture content but highest percentage of ethanol while sample B has the highest percentage of moisture content with the least percentage of ethanol using the same quantity of sample i.e. (100g each).this may be attributed to the sample's low acid content compared to samples B and C both having orange with high citric acid content, The concentration of bioethanol in sample A can be due to the effective activity of the sacchromyces cerevisiae as enzymes for the production of ethanol which is similar to the work of Talebnia, (2008).

Table 3: Percentage of moisture content and bioethanol from each sample during first stage of distillation

Sample	Mass (g)	Volume of ethanol – water mixture (ml)	Moisture content (%)	Ethanol (%)
A(mango waste)	100	590	23.73	76.27
B(orange waste)	100	521.6	29.00	71
C(mango and orange)	100	575.5	25.20	74.8

The result of Table 4 and 5 represent the density of ethanol at first and second stage of distillation which show that sample A of Table 4 has the least density of 8.48 kg/m^3 with a volume of 5.9 x 10^{-4} m^3 while sample C has the highest density of 9.43 kg/m^3 with a volume of 6.1 x 10^{-4} m^3. Similarly, in Table 5 the volume of each sample decreases and the density increases, sample also decreases. This shows that sample A is less dense and has 793.30 kg/m^3 with 4.5 x 10^{-4} m^3 while sample C is denser and has 813 kg/m^3 with 4.3 x10^{-4} m^3, this confirms that the density of sample C is as a result of it mass and volume in both first and second distillation as clearly shown in both Tables.

Table 4: Density of ethanol- water mixture of each sample at first stage distillation

Sample	Mass (m) (kg)	Volume (V) (m^3)	Density (ℓ) (kg/m^3)
A(mango waste)	0.5006	5.9 x 10^{-4}	8.48
B(orange waste)	0.5216	5.8 x 10^{-4}	8.99
C(mango and orange)	0.5755	6.1 x 10^{-4}	9.43

Table 5: Density of ethanol at second stage of distillation

Sample	Mass (M_2) (kg)	Volume (V_2) (m^3)	Density (ℓ_2) (kg/m^3)
A(mango waste)	0.357	4.5 x 10^{-4}	793.30
B(orange waste)	0.296	3.7 x 10^{-4}	800.00
C(mango and orange)	0.350	4.3 x 10^{-4}	813.00

The yield result of the composition showed an increase in the ethanol production (Table 6) for sample A (rotten mango) and decrease in sample C (orange and mango). The fermentation of waste fruits by saccharomyces cerevisiae for ethanol production had a high percentage of ethanol. For this research the overall percentage of ethanol produced did not exceed 19.98% for sample A(waste mango), 19.17% for sample B (orange) and 17.38% sample C (orange and mango). The yield which is generally low is due to small amount of sample used.

Table 6: Percentage of ethanol yield from each sample used

Sample	Mass (g)	Volume (ml)	Yield point (%)
A(mango waste)	100	500.6	19.98
B(orange waste)	100	521.6	19.17
C(mango and orange)	100	575.5	17.38

Table 7 shows the results of the estimated mean properties of the Bioethanol investigations carried out with respect to waste fruits of mango and orange as the feed stock.

The results in table 7 were further subjected to statistical analysis (ANOVA) using Duncan Multiple Range Test (DMRT) of the fuel properties of ethanol samples. It is obvious from the

result in table 8 that the observed differences were not significant, however, the mean treatment difference observed between sample 1 and 4, sample 2 and 4, and sample 3 and 4 for the flash point were significant at 5% level. This is probably due to high moisture present in the three samples compared with the control sample (sample 4). This is responsible for the lower flash point of sample 4 compared to that of samples 1, 2 and 3 in Table 7.

Table 7. Estimated Mean of Ethanol Properties

	Flash point	Boiling Point	Viscosity	pH	Refractive Index	Relative Density	Specific gravity
1-Ethanol from MANGO	28.6	79.6	1.4	5.3	1.36	0.91	0.79
2- Ethanol from ORANGE	30.2	81.3	1.8	4.8	1.35	0.97	0.8
3-Ethanol from MANGO/ORANGE	29.7	83.5	1.7	3.9	1.34	0.96	0.81
4-ETHANOL	12.2	78.3	1.09	5.2	1.35	0.78	0.79

Table 8. Mean Treatment Difference of Ethanol Properties

	Flash Point	Boiling Point	Viscosity	pH	Refractive Index	Relative Density	Specific gravity	Df
1 vs 2	-1.6	-1.7	-0.4	0.5	0.01	-0.06	-0.01	1
1 vs 3	-1.1	-3.9	-0.3	1.4	0.02	-0.05	-0.02	1
1 vs 4	16.4	1.3	0.31	0.1	0.01	0.13	0	1
2 vs 3	0.5	-2.2	0.1	0.9	0.01	0.01	-0.01	1
2 vs 4	18	3	0.71	-0.4	0	0.19	0.01	1
3 vs 4	17.5	5.2	0.61	-1.3	-0.01	0.18	0.02	1

Where 1= Ethanol from Mango; 2= Ethanol from Orange; 3= Ethanol from Mango/orange; 4=Ethanol

4.0 Conclusion

Bio-ethanol has been extracted from waste fruits of mango and orange in equal proportion through fermentation process (sample A, B and C). Sample A (mango waste fruit) was identified as the feedstock with the highest yield of 19.98% among the Three samples. Sample B (orange waste fruit) yielded 19.17% while sample C (mango and orange waste fruits in equal proportion) yielded 17.38% of bio-ethanol. The properties of extracted bioethanol were compared with standard ethanol, the properties showed similarities within 5% (average error) when analysed using the Duncan multiple range test (DMRT) in design expert 7.0 statistical package, which was in agreement with that of pure ethanol requiring 5-10% gasoline addition for de-naturing before use. Furthermore, ethanol at the first stage of distillation produced should be subjected to another distillation or separation method such as fractional distillation, membrane separation etc, this is to refine the process in order to separate ethanol from water for absolute combustibility.

The study further shows that waste fruits (mango and orange) can be put to use by increasing the scale of use of these waste material for bio-ethanol production. It also minimizes the economic loss that may be accrued to the waste fruits and by extension contributes immensely to carbon sequestration.

For further research, more varieties of waste fruits should be used so as to know which variety will produce ethanol optimally.

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